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THE SIGNIFICANCE OF THE CAUDAL EPIDERMIS IN ASCIDIAN METAMORPHOSIS[1]

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Free swimming ascidian larvae normally attach to solid substrata before the onset of metamorphosis. Morphogenetic changes include tail resorption, reorientation of the digestive system, heart and adult neural tissues (or their primordia) and the formation of epidermal ampullae. Degeneration of the larval nervous and sensory systems and the loss of the outer layer of tunic follow rapidly.

The most astonishing event in metamorphosis is the withdrawal of the tail. In the simple ascidians, *Boltenia villosa, Pyura haustor, Styela gibbsii,* and *Styela partita,* the major part of tail resorption is completed within 10 minutes. In the compound ascidian *Amaroucium constellatum* the tail is completely withdrawn into the posterior region of the trunk in about 6 minutes. Histological studies of *Boltenia, Pyura* and *Styela* (Cloney, 1961a) have demonstrated that a disruption of the intercellular cementing substances or binding forces between the notochordal and muscle cells occurs during tail resorption. These disruptive changes begin proximally and progress distally as the tail shortens. The anterior end of the notochordal sheath first explosively ruptures. This allows the notochordal cells and the extracellular matrix of the notochord to flow into the posterior region of the trunk. Simultaneously the muscle cells buckle and their myofibrils become disarranged and are no longer oriented parallel to the axis of shortening. The nerve cord and endodermal strand are passive and become compressed as the tail shortens. The epidermis thickens but shows no signs of dissociating into separate cells. This pattern of tail resorption will be referred to as Type 1.

In *Ciona intestinalis* (Weiss, 1928), *Phallusia mammillata, Clavelina lepidiformis* (Berrill, 1947), *Amaroucium constellatum* (Scott, 1952), *Ascidia nigra* (Grave, 1935) and *Ascidia callosa* (Cloney, 1961a), the notochord-muscle-nerve cord complex (NMN-complex) remains intact as a unit as the caudal tissues are withdrawn. The NMN-complex separates from the epidermis and moves into the

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posterior region of the trunk. In some species the complex is formed into a helix as it moves. The epidermis finally forms a cap over the resorbed tissues. This will be called Type 2 tail resorption.

When tails of *Boltenia* larvae were excised before the onset of metamorphosis, the distal fragments remained alive and were observed to twitch or even swim about for one or two days after the operation, but they showed no signs of the histological changes associated with tail resorption. Connection with the trunk is normally essential for these changes.

If, however, the tails were excised through the proximal region of shortening after the onset of metamorphosis they could undergo shortening in isolation. Evidently the mechanism for tail resorption resides within the tail tissues after the process has begun. Isolated tail can also be induced to shorten with proteolytic enzymes.

Instructive cases of partial tail resorption in chloretone-treated *Ciona* larvae have been observed by Weiss 1928). In these specimens the NMN-complex remained rigid but the epidermis sometimes became torn or ruptured in places and pulled into a mass near the tip of the tail. Weiss emphasized that the epidermis can therefore undergo typical regressive changes by itself. Direct observations of tail resorption, however, led him to conclude that both epidermal and tonic muscular contraction are probably essential for complete tail involution in *Ciona*.

Berrill (1947) contended that tail resorption is caused principally by the shrinkage of the epidermis due to its so-called "nutritional exhaustion" in *Ciona, Ascidiella, Phallusia, Styela, Styelopsis, Distomus, Stolonica, Clavelina* and *Distaplia*. This opinion was supported by Scott (1952). Cloney (1961a) argued that the epidermis is an active tissue, as evidenced by (1) the resorption of the epidermal adhesive papillae within two to three minutes after the onset of metamorphosis in *Boltenia, Pyura, Styela* and *Ascidia;* (2) the formation of an invagination of the epidermis behind the resorbed tail tissue elements at the end of tail resorption, and (3) the rapid formation of the epidermal ampullae which spread out over the substratum shortly after attachment of the larva. There is no evidence of "nutritional exhaustion" in these species; indeed, the epidermal cells display considerable activity and they also contain yolk granules. (For a fuller discussion of this point see Cloney, 1961a.)

It was suggested that the release of a proteolytic enzyme in the posterior region of the trunk in *Boltenia* could account for the rupture of the notochord, the dissociation of the muscle and notochordal cells, and that the epidermis contracts actively as a unit, forcing the dissociated tissues into the trunk.

But, since in *Boltenia, Pyura,* and *Styela,* it was not possible to isolate or to observe the independent contraction of the tail epidermis, only indirect evidence for its active role in the resorption of the tail could be obtained. Consideration of Type 1 and Type 2 tail resorption led to the following questions: (1) Does contraction of the muscle cells play any part in the process? All analyses of tail resorption have shown that the epidermis is important, but (2) what is the mechanism of epidermal contraction? (3) What factors initiate and synchronize the observed histological changes with general metamorphosis? (4) Can the two distinct types of tail resorption be explained with a single hypothesis? This report on the compound ascidian, *Amaroucium constellatum,* provides direct evidence for:

(1) the active contraction of the epidermis in unaesthetized larvae, (2) the insignificance of the muscle cells in the overall mechanism of tail resorption, (3) a possible explanation for other histological changes in the caudal tissues of ascidians during tail resorption.

Methods

Observations were made on larvae of the compound ascidian *Amaroucium constellatum* collected by the Supply Department in the vicinity of the Marine Biological Laboratory, Woods Hole, Massachusetts. Colonies were kept in running sea water in a light-tight box for 8 to 18 hours prior to the period when they were needed. Colonies were then exposed to light in a small dish of sea water. This method, used by Scott (1952), induces the release of swimming larvae in about 20 minutes. Observations of living animals were made with and without supported coverslips under a compound microscope equipped with NA 0.25 and 0.65 objectives. In some cases metamorphosis was stimulated with a 1:500,000 dilution of Janus green B. Cinematographic records were made of both normal tail resorption and the effects of experimental interference with tail resorption. Tissues were fixed in 2.7% OsO_4 buffered in 0.1 *M* *S*-collidine and embedded in Epon, according to the method of Luft (1961). Sections were cut at 1 μ and stained with Richardson's stain (Richardson *et al.*, 1960). Photomicrographs of sections were made with a Zeiss NA 1.25 planachromat objective.

Results

A. *Structure of the larval tail*

Details of the larval anatomy have been described by Grave (1921) and Scott (1946). The following descriptions of the tail tissues are limited to details regarded as essential to a discussion of tail resorption.

Tunic. The entire trunk and tail of the larva is covered by two tough membranous layers of tunic. The outer layer forms the dorsal and ventral fins and is lost as a molt during metamorphosis. The inner layer is retained as part of the post-larval tunic. Free amoeboid cells are frequently found between the layers (Fig. 1). The fine structure of this complex structure will be described in a subsequent paper.

Epidermis. The epidermis is a simple squamous epithelium supported basally by a thick amorphous basement membrane which lies in close contact with the surfaces of the underlying muscle cells. In transverse sections of the tail the epidermis consequently tends to conform to the scalloped contours of the muscle bands (Fig. 1).

Muscle. Four rows of muscle cells are arranged in bands on the right and left sides of the tail. (Since the tail is twisted 90° to the left during development, the top of the tail is considered to represent the anatomical right side and the bottom the left side. The dorsal and ventral fins lie in the frontal plane.) Within each row, the muscle cells abut against each other at their ends without the intervention of a connective tissue septum. Each muscle cell is roughly cylindrical in shape. Contractile myofibrils lie immediately beneath the sarcolemma in a single layer. They spiral along the course of the cell at an angle of about 18° to the right (Grave, 1921). They are disposed entirely around the periphery of each cell except for

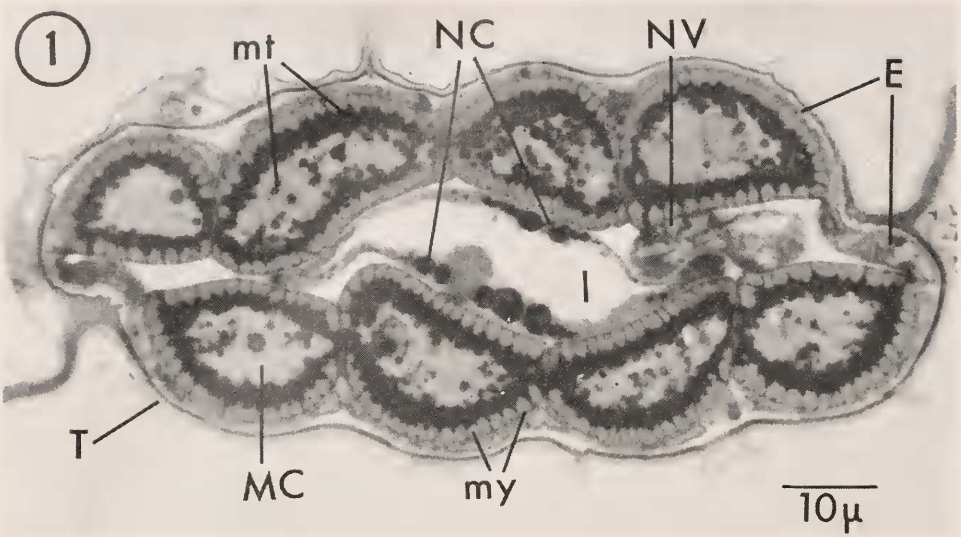


FIGURE 1. *Amaroucium constellatum;* transverse section of the larval tail. The tail is covered by two layers of tunic (T). The outer layer forms the dorsal and ventral fins. The basement membrane of the thin epidermis (E) lies in close contact with 8 rows of muscle cells (MC). Myrofibrils (my) lie in the periphery of each cell. They are underlain by a thick layer of mitochondria (mt). The muscle cells attach to a fibrous sheath of the notochord. Notochordal cells (NC) form a squamous epithelium beneath the sheath. The axis or lumen (1) of the notochord is filled with a clear matrix. The nerve cord (NV) is visible on the right. One-micron Epon section; Richardson's stain.

small gaps where adjacent muscle cells lie in close contact. Contrary to the opinion of Grave (1921), Scott (1946) and Berrill (1947), the myofibrils terminate near the sarcolemma at the ends of each cell (Jackson, 1958; Cloney, unpublished results). A dense layer of mitochondria is located beneath the myofibrils. The nucleus is located near the center of each cell and is surrounded by relatively clear cytoplasm. Lead acetate staining of ultrathin sections suggests the presence of glycogen in these areas. Large, irregular, dense bodies are often found in the cytoplasm of the muscle cells. In electron micrographs these bodies can be seen to contain myelinic figures. The band of muscle cells on the right side of the tail is often shifted slightly with respect to the left band of muscle, giving the tail an asymmetric appearance in section (Fig. 1).

Notochord. The notochord forms the axis of the tail. In transverse sections the notochord appears elongate in the dorsal-ventral axis and somewhat irregular in shape. In electron micrographs, it is seen to be surrounded by an acellular filamentous sheath. The cells of the notochord are arranged in an epithelium and are attached to the inside of the notochordal sheath. The cells contain both proteid and lipid yolk granules. The axis or lumen of the notochord is filled with a clear matrix. The matrix stains poorly and has low electron density. Processes frequently extend from the notochordal cells into the lumen (Figs. 1 and 2).

Nerve cord. The dorsal nerve cord has a well formed lumen in many places. The cord extends posteriorly from the visceral ganglion into the dorsal region of

the tail. Nerve processes have not yet been observed passing from the cord to the muscle cells (Fig. 1).

B. *Tail resorption*

The basic morphological changes accompanying metamorphosis in *Amaroucium* have been described by Scott (1952). Only the details of tail resorption will be considered here.

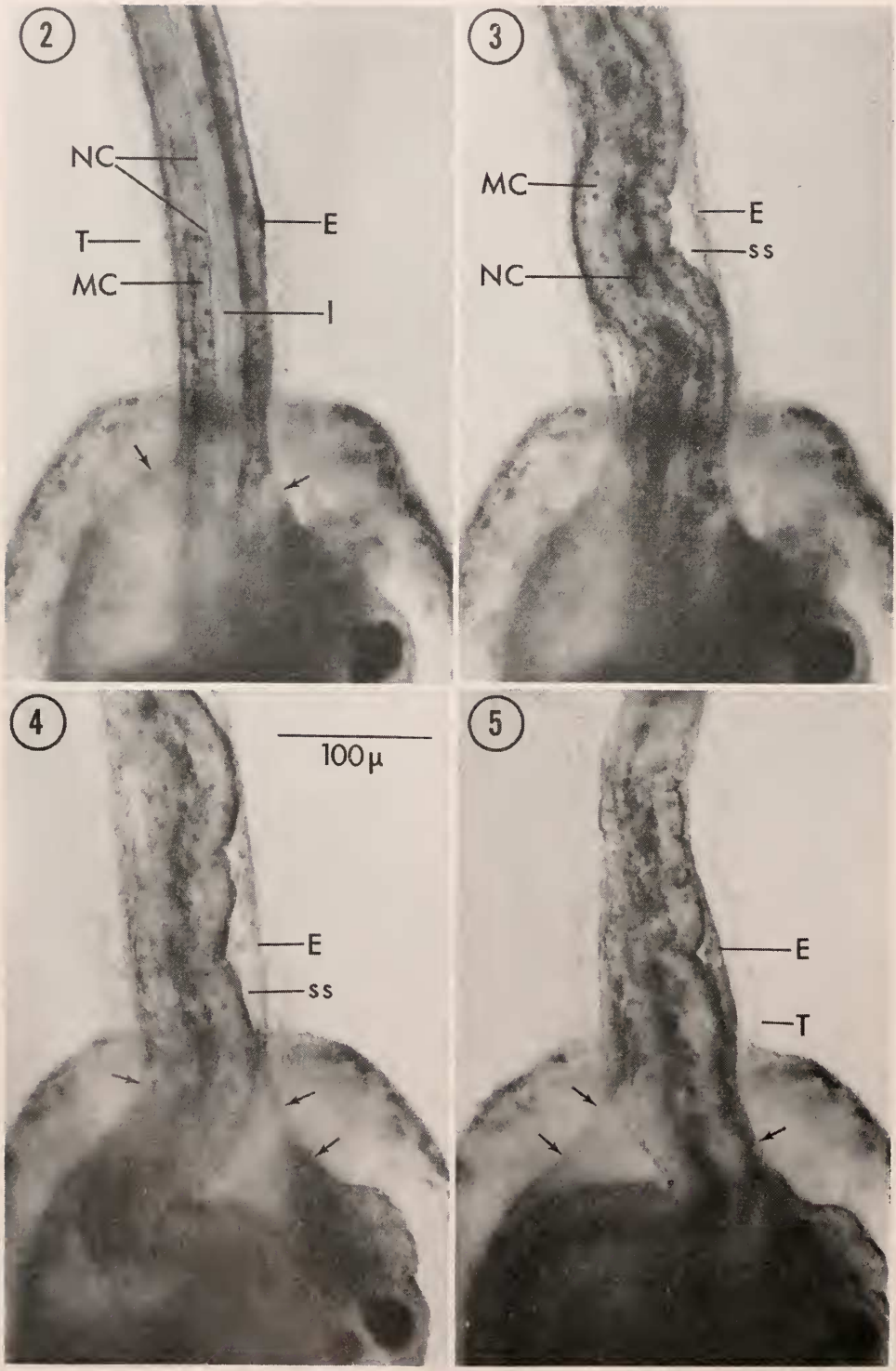
In 14 recorded cases, the first detectable morphological changes associated with tail resorption occurred on an average of two minutes, two seconds after the discharge of the larval adhesive papillae (see Table I). The discharge of a sticky substance by the papillae is typically associated with attachment in *Amaroucium,* but metamorphosis may ensue with or without attachment. Metamorphosis may be spontaneous or it may be induced by a heterogeneous variety of substances (Lynch, 1961). Changes in the tail tissues begin with the lifting away of the epidermis from the underlying notochord-muscle-nerve cord (NMN) complex with the formation of a subepidermal fluid-filled space (Figs. 3, 6). Simultaneously the notochord loses its turgidity. This results in a partial collapse of the tail (Fig. 3). The notochordal cells begin to round up and large vesicles sometimes become visible within the matrix of the notochord (Fig. 6). Under favorable optical conditions these vesicles can sometimes be seen to flow toward the trunk. The entire NMN-com-

TABLE I

Timing of tail resorption.

The first sign of the onset of metamorphosis in A. constellatum is the discharge of the adhesive papillae. In column I, tabulations indicate the elapsed time in minutes and seconds from the discharge of the adhesive papillae to the first visible changes in the tail. Column II indicates the time elapsed from the earliest changes in the tail to the completion of tail resorption. Column III indicates the total elapsed time from the discharge of the adhesive papillae to the completion of tail resorption. Measurements were made at 24.5°C. Tabulations of 14 separate cases were arranged in order of increasing total time.

Specimen	Discharge of papillae to beginning of tail resorption I	Beginning to completion of tail resorption II	Total time III
1	2′20″	4′00″	6′20″
2	1′10″	5′40″	6′50″
3	2′15″	5′00″	7′15″
4	1′56″	5′37″	7′33″
5	1′50″	5′45″	7′35″
6	2′25″	5′10″	7′35″
7	1′25″	6′20″	7′45″
8	2′42″	5′05″	7′47″
9	2′30″	6′00″	8′30″
10	2′45″	5′50″	8′35″
11	1′45″	6′50″	8′35″
12	2′05″	6′32″	8′37″
13	2′00″	6′45″	8′45″
14	1′21″	7′53″	9′14″
Average	2′02″	5′54″	7′55″


2
NC
T
MC
E
I
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MC
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4
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5
E
T

plex beneath the epidermis begins to buckle and fold as it moves into the posterior region of the trunk (Figs. 3, 4, 5). The epidermis continuously thickens and appears to be under tension. The epidermis finally forms into a thick cap at the posterior end of the trunk, enclosing the entire NMN-complex within the body cavity.

As the tail tissues are withdrawn, the double-layered tunic which covers the tail becomes greatly folded. Near the completion of tail resorption the outer layer of tunic springs away from the underlying tissues and is pushed out, forming an empty sac. This outer layer drops away within a few hours as a cuticular molt. This outer layer need not be regarded as important to tail resorption because it can be pulled off with a pair of forceps before metamorphosis begins without affecting the process in any way.

In observing tail resorption, one might infer that the epidermis is actively contracting because it appears to be under tension during this period, but it is of course impossible to be certain of this from microscopic observations alone.

To test the theory of active epidermal contraction, the tails of larvae *which had just begun to metamorphose* (after the epidermis has separated from the underlying NMN-complex) were either excised about halfway along their length or they were regionally damaged by touching them with a needle. When this was done the epidermis immediately split into two units: an isolated distal piece and a proximal piece continuous with the trunk epidermis. *Immediately following this operation both the proximal and the distal pieces of epidermis began to shorten over the surface of the underlying NMN-complex* (Figs. 7–10). *Sometimes the epidermis contracted, pulling the underlying tissues with it for a short distance, and then the NMN-complex, evidently under tension, broke loose and straightened out again while the epidermis continued to contract.* The proximal epidermis shortened into a thick annular ring at the base of the tail while the distal fragment pulled distally, forming a thickened cap of epithelium around the distal segment of the NMN-complex. This experiment was repeated several dozen times with the same results.

In this experiment the epidermis manifests its capacity to shorten independently

FIGURE 2. *Amaroucium constellatum;* right side of living larva. The epidermis (E), muscle cells (MC), notochordal cells (NC), notochordal lumen (I) and the tunic (T) are visible. Note the squamous epithelium within the notochord. Arrows indicate the position of the epidermis within the trunk.

FIGURE 3. *Amaroucium constellatum;* right side of same specimen as Figure 2, about one minute after the onset of tail resorption. Note the appearance of a space (ss) beneath the epidermis and the marked change in the arrangement and shape of the notochordal cells (NC). The epidermis (E) has separated from the surface of the muscle cells (MC) and has begun to thicken. The NMN-complex has begun the fold as a unit without a breakdown of the binding force or cementing substances between cells.

FIGURE 4. *Amaroucium constellatum;* right side of another specimen about two minutes after the onset of tail resorption. The NMN-complex has become more folded and has been partially forced into the posterior end of the trunk. The epidermis (E) has become thicker than it was in the larva.

FIGURE 5. *Amaroucium constellatum;* right side of same specimen as seen in Figure 4 about three minutes after the onset of metamorphosis. More than half of the tail is coiled within the posterior end of the trunk. The epidermis is greatly thickened.

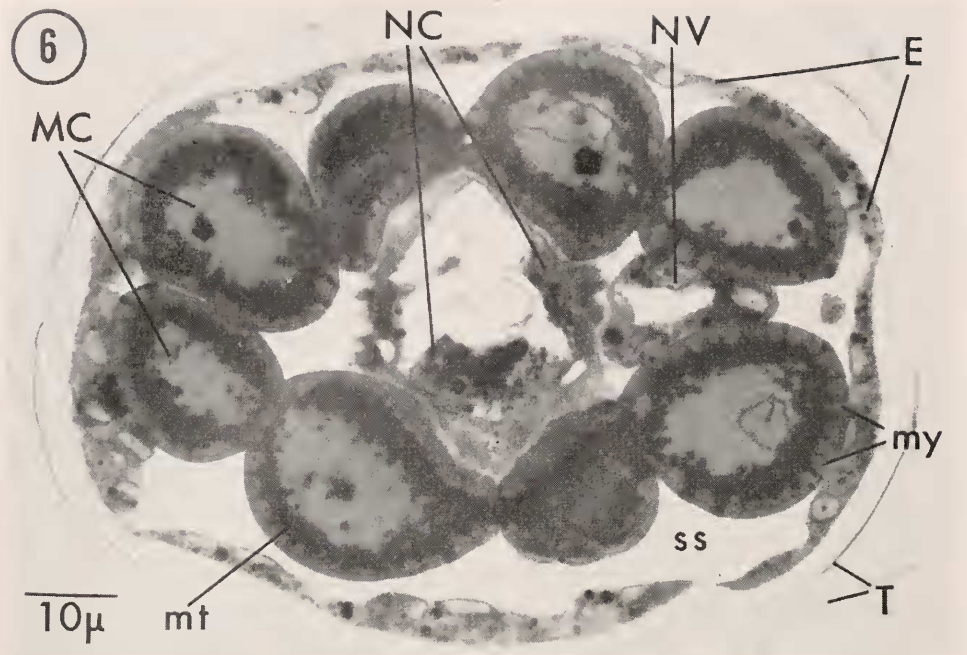


FIGURE 6. *Amaroucium constellatum*. Transverse section of the tail about two minutes after the onset of metamorphosis. A subepidermal space (ss) is prominent. The epidermis (E) is thicker than in the larva. Marked changes are apparent in the notochord. The cells (NC) tend to round up, filling the lumen. Many large vesicles are formed. The muscle cells (MC) increase somewhat in diameter as the NMN-complex bends and folds. One-micron Epon section; Richardson's stain.

of the other tissues. The NMN-complex is left bare by this action (except for the tunic which covers the entire organism). Under these circumstances the NMN-complex is never withdrawn into the trunk; the muscle cells do not manifest any capacity to shorten. These tissues remain unresorbed while the rest of the larva completes its metamorphosis.

Tails excised before the onset of metamorphoses do not undergo any of the characteristic histological changes observed in normal metamorphosing larvae or in tails excised after the onset of tail resorption. They twitch for many hours but eventually degenerate. The tail tissues are not necessary for post-larval development in *Amaroucium* (Scott, 1952), or in *Boltenia* (Cloney, 1961b). The caudal tissues have no known prospective significance but probably serve a nutritive function.

This rupturing of the caudal epidermis occurs spontaneously in some larvae collected in culture dishes. Numerous cases have been observed in which metamorphosis proceeded in the trunk without the resorption of the tail. This has also been reported by Scott (1952). Close inspection of more than a dozen of these specimens which failed to resorb their tails revealed that in all cases, there was a mass of epidermis at the base of the tail and at the tip of the tail, while the central portion of the tail was not covered by epidermal tissue.

Failure to resorb the tail may be attributed, at least in these cases, to the rupture of the epidermal envelope which normally contracts and is essential to the withdrawal of the NMN-complex.

The question of the energetics of contraction remains to be considered. The epidermis might shorten through elastic properties or it could actively contract and be dependent on aerobic oxidative processes for energy. The latter is suggested by the following experiment.

After the beginning of metamorphosis and following the onset of tail resorption, when the epidermis of the tail has separated from the underlying muscle, the larvae were placed for varying periods in a solution of 5×10^{-3} to 1×10^{-2} *M* KCN in sea water regulated to pH 8.0 with HCl (Fig. 11). Within about one minute after exposure to KCN the rate of shortening was slowed down. If the specimens were then washed in sea water, tail resorption would resume after a minute or two. Tail resorption is thus reversibly inhibited by potassium cyanide. Sodium azide has a similar inhibitory effect in a concentration of 10^{-2} *M*. Both of these substances have been reported to reversibly inhibit the *onset* of metamorphosis in *Amaroucium* larvae (Lynch, 1961). Potassium cyanide and sodium azide are well known for their inhibitory affects on cytochrome oxidase, the terminal enzyme complex in the respiratory chain (Wainio and Cooperstein, 1956; Pearse, 1960).

Discussion

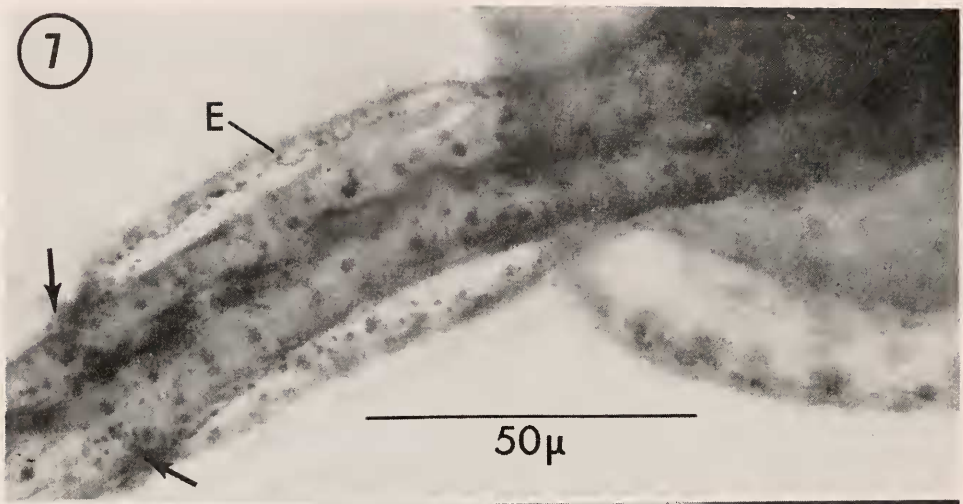
The overall aspects of tail resorption in *Amaroucium* correspond to Type 2 as mentioned in the introduction. This pattern of tail resorption has features in common with Type 1.

In both forms there are changes in the notochord. The notochordal matrix which contributes to the rigidity of the tail (hydrostatic skeleton) is released and leads to a loss of turgor and a partial collapse in both forms. This aspect of tail resorption was not reported by Weiss (1928) in his study of *Ciona*. In *Boltenia*, *Styela* and *Pyura*, both the muscle cells and notochordal cells become detached from the notochordal sheath as the tail is withdrawn. This process does not occur in *Amaroucium*, or other Type 2 forms. The muscle cells and notochordal cells remain associated in the same linear relationships, attached to the notochordal sheath. But in *Amaroucium* there is a rapid dissociation of the epidermis from the underlying muscle cells, and rapid changes in the notochordal cells.

The activity of a proteolytic enzyme might account for changes in the notochord and a breakdown of adhesion between tissues in all species. Conklin (1931) has made a similar suggestion to explain tail resorption in *Styela partita* but no direct evidence for the existence of a proteolytic or hydrolytic enzyme that is released or is activated at the time of metamorphosis has yet been found.

It seems reasonable, in view of the experiments with *Amaroucium* and the evidence of epidermal activities in *Ciona* (Weiss, 1928), and *Boltenia*, to generalize the statement that the contraction of the epidermis is responsible for forcing the tissues of the tail into the trunk at the time of tail resorption in ascidians.

Weiss' contention that tonic muscular contraction must contribute to the withdrawal of the tail seems untenable for the following reasons: (1) In Type 1 tail resorption the muscle cells become dissociated, and histological analyses show that the contractile elements become disarranged early in tail resorption. (2) In Type 2 tail resorption excision of the tail after the beginning of tail resorption leads to


7
E
50μ


8
E


9
MC
I

the retraction of the epidermis alone, while the NMN-complex actually pushes away from the trunk as if released from tension as soon as it detaches from the epidermis.

The mechanism of epidermal contraction is of general biological interest. The following is quoted from Hoffmann-Berling (1960, p. 346).

"All in all, it may be stated that systematic comparisons have not yet uncovered any dissimilarity which would indicate a fundamental difference between the contraction of a muscle and the contraction of an undifferentiated cell. The differences are only quantitative. The mechanism of muscle contraction is already present in the final form at the developmental stage of the single cell organism prior to the start of organ formation and tissue specialization. It is older than muscle itself."

Unpublished electron micrographs revealed filaments in the epidermis of *Amaroucium* larvae, but filaments are commonly found in epidermal cells, as well as many other epithelial cells in which active contraction is at least not obvious. Contractility may be one of the fundamental properties of all cells but in the case of tail resorption, some cells contract in a specific and predictable way while other cells of the tail do not perceptibly contract. These epithelial cells may be suitable subjects for further investigations of this long-standing problem.

Summary

1. Tail resorption in *Amaroucium* is a rapid morphogenetic process. It is usually complete within only 6 minutes.

2. The initiation of tail resorption is signaled by a rapid separation of the epi-

Figures 7–10. *Amaroucium constellatum;* sequence of events following the excision of the distal one-third of the tail; living specimen. Immediately after the beginning of tail resorption the tail was excised and photographed. The epidermis began to shorten by contracting over the surface of the underlying NMN-complex. Within two minutes the epidermis shortened into an annular ring at the base of the tail. The NMN-complex invariably fails to be resorbed in this experiment. The epidermis of the distal segment (not shown) also shortens over the underlying tissues following excision. The arrows indicate the cut surface of the epidermis through stages of contraction.

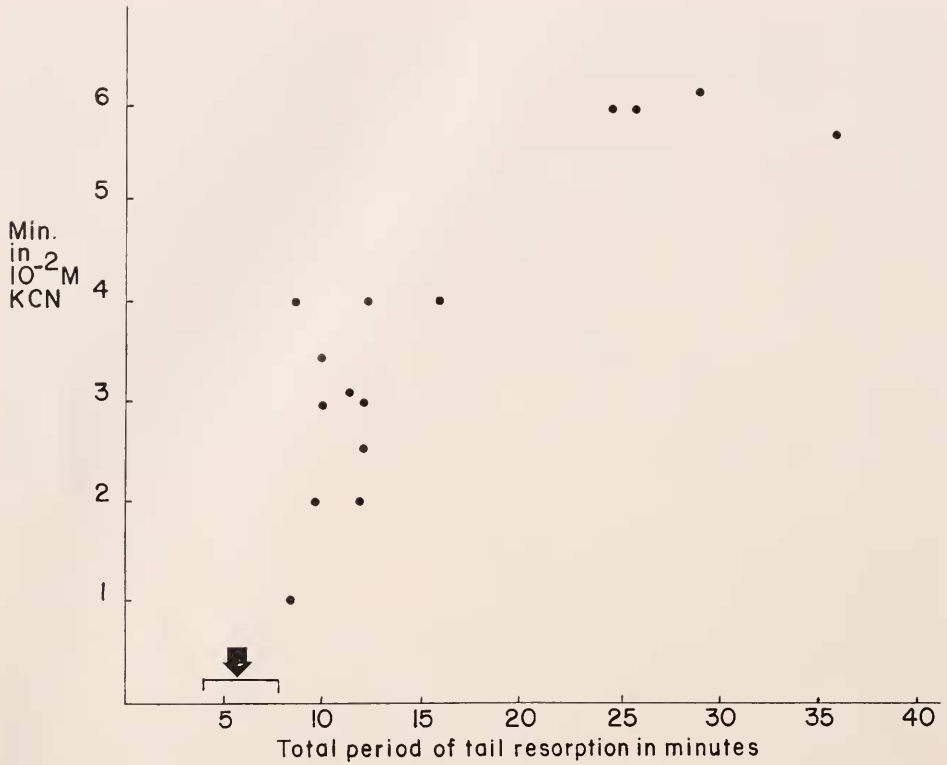


FIGURE 11. Inhibition of tail resorption by potassium cyanide. The total time required for the completion of tail resorption was plotted for larvae treated for various times in a solution of 10^{-2} *M* KCN. Experimental larvae were first observed until the first signs of tail resorption could be detected. They were then transferred to the KCN solution for from 1 to 6 minutes. They were subsequently washed in sea water. Cyanide treatment slows down the rate of tail resorption beyond the controls. The range of normal tail resorption is shown by a line on the lower left of the graph. The arrow indicates the average time of tail resorption in 14 normal specimens.

dermis of the tail from the underlying notochord-muscle-nerve cord complex (NMN-complex). This results in the formation of a fluid-filled subepidermal space. The NMN-complex buckles and folds as it moves into the posterior end of the trunk. The epidermis forms a thickened cap over the end of the trunk, enclosing the other tail tissue.

3. When the tail was excised after the beginning of tail resorption, the epidermis was observed to retract independently of the other tail tissues.

4. The muscle cells do not manifest any tendency to shorten without the epidermis.

5. Potassium cyanide and sodium azide reversibly inhibit the onset of metamorphosis and slow down the rate of tail resorption if they are applied after the beginning of metamorphosis.

6. Some histological changes in the tail of *Amaroucium* and other species of ascidians may be the result of the activity of a proteolytic enzyme.

LITERATURE CITED

Berrill, N. J., 1947. Metamorphosis in ascidians. *J. Morph.*, **81**: 249–267.

Cloney, R. A., 1961a. Observations on the mechanism of tail resorption in ascidians. *Amer. Zool.*, **1**: 67–87.

Cloney, R. A., 1961b. Changes in the fine structure of striated muscle cells during metamorphosis in ascidian larvae. *Anat. Rec.*, **139**: 217–218.

Conklin, E. G., 1931. The development of centrifuged eggs of ascidians. *J. Exp. Zool.*, **60**: 1–119.

Grave, Caswell, 1921. *Amaroucium constellatum* (Verrill) II. The structure and organization of the tadpole. *J. Morph.*, **36**: 71–91.

Grave, Caswell, 1935. Metamorphosis of ascidian larvae. Papers from the Tortugas Lab. *Carn. Inst. Wash. Publ., No.* **452**: 209–292.

Hoffmann-Berling, Hartmut, 1960. Other mechanisms producing movements. *In:* Comparative Biochemistry, Marcel Florkin and Howard S. Mason, editors. Vol. 2, pp. 341–370. Academic Press, New York.

Jackson, Sylvia Fitton, 1958. Some aspects of morphogenesis in ascidians. *Biol. Bull.*, **115**: 335.

Lynch, W. F., 1961. Extrinsic factors influencing metamorphosis in bryozoan and ascidian larvae. *Amer. Zool.*, **1**: 59–66.

Luft, John, 1961. Improvements in epoxy resin embedding methods. *J. Cell Biol.*, **9**: 409–414.

Pearse, A. G. E., 1960. Histochemistry. Little, Brown and Co., Boston.

Richardson, K. C., L. Jarett and E. H. Finke, 1960. Embedding in epoxy resins for ultrathin sectioning in electron microscopy. *Stain Tech.*, **35**: 313–323.

Scott, Sister F. M., 1946. The developmental history of *Amaroecium constellatum*. II. Organogenesis of the larval action system. *Biol. Bull.*, **91**: 66–87.

Scott, Sister F. M., 1952. The developmental history of *Amaroecium constellatum*. III. Metamorphosis. *Biol. Bull.*, **103**: 226–241.

Wainio, W. W., and S. J. Cooperstein, 1956. Some controversial aspects of mammalian cytochromes. *Adv. in Enzym.*, **17**: 329–392.

Weiss, Paul, 1928. Experimentelle Untersuchungen über die Metamorphoses der Ascidien II. Versuche über der Mechanismus der Schwanzinvolution. *Biol. Zentr.*, **48**: 387–407.